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Impact of starch-based emulsions on the antibacterial efficacies of nisin and thymol in cantaloupe juice

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ABSTRACT

The use of antimicrobial compounds to prevent foodborne pathogens from contaminating fresh-cut produce has received broad attentions; however, the applications of these compounds are hindered by their rapid depletion in foods. To prolong their efficacies, the use of delivery systems is essential. In this study, oil-in-water emulsions formed using starch octenyl succinate (starch-OS) were used to stabilize nisin and thymol in cantaloupe juice-containing fluid. *Listeria monocytogenes* V7 and *Salmonella enterica* serovar Typhimurium were used as model pathogens to evaluate the antimicrobial activities of nisin and thymol formulations in cantaloupe juice. The results showed that the emulsions had much greater capability to retain nisin and thymol over the storage and displayed much greater effect to inhibit *Listeria* and *Salmonella* than non-emulsion, aqueous formulations. Starch-OS based emulsions not only retained nisin and thymol activities separately, but also exhibited their cooperative antibacterial effects.

1. Introduction

Cantaloupe has an important economic role among fresh and fresh-cut produce, and it is also a primary source for human pathogen outbreak (Martiñon, Moreira, Castell-Perez, & Gomes, 2014). Cantaloupe has been associated with contaminations of various pathogenic organisms such as *Listeria* and *Salmonella enterica* (Fang, Liu, & Huang, 2013; Ismail, Chan, Mariod, & Ismail, 2010; Mahmoud, 2012). Various thermal and non-thermal processing techniques are utilized for improving the shelf-life of fresh-cut fruits (Martiñon et al., 2014). Meanwhile, the food industry has constantly been exploring novel and cost-effective technologies for minimally processed foods. For fresh and fresh-cut produce including cantaloupes, the use of natural antimicrobial compounds can be a feasible approach.

Natural antimicrobials such as essential oils (e.g. thymol) and bacteriocins (e.g. nisin) have strong antibacterial efficacies and are also generally considered as safe for food application (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Burt, 2004). Nisin is an antimicrobial peptide generated by *Lactococcus lactis* and inhibits Gram-positive bacteria through pore-forming on cytoplasmic membrane. In general, it is ineffective against Gram-negative bacteria (Breukink & de Kruijff, 2006; Breukink, Ganz, De Kruijff, & Seelig, 2000). Thymol (2-isopropyl-5-methylphenol) is a natural phenolic compound found in thyme (*Thymus vulgaris*) essential oil and has shown inhibitory effects against both Gram-positive and negative bacteria (Burt, 2004). The antibacterial efficacy of thymol is due to the interactions between its phenolic structure and the phospholipids within the cytoplasmic membrane (Burt, 2004).

However, antimicrobial compounds may undergo quick depletion within foods. For example, nisin is susceptible to depletion due to its physical diffusion within the food system or degradation by proteases. In addition, components within foods such as glutathione, sodium metabisulphite, and titanium dioxide can react with nisin, thereby reducing its effective concentration (Bhatti, Veeramachaneni, & Shelef, 2004; Jung, Bodyfelt, & Daeschel, 1992; Mahadeo & Tatini, 1994; Rose, Sporns, Stiles, & McMullen, 1999). Thymol is chemically stable, however, it may be easily lost due to evaporation. Thymol may also interact with hydrophobic food components, which reduce its concentration in the aqueous phase (Shah, Davidson, & Zhong, 2012; Shah, Davidson, & Zhong, 2013). To protect antimicrobials from depletions in foods, the use of protective delivery agents could be very beneficial.

Delivery system of different types have been designed for the protection of antimicrobial compounds. For example, antimicrobial molecules within essential oils have been encapsulated within protein or polysaccharide carriers (Baranauskienė, Venskutonis, Dewettinck, & Verhé, 2006; Beristain, Garcıa, & Vernon-Carter, 2001) and emulsions (Suriyarak & Weiss, 2014). Different matrices

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such as β-cyclodextrin (Cevallos, Buera, & Elizalde, 2010), zein nanoparticles (Zhang et al., 2014), whey protein isolate-maltodextrin conjugates (Shah, Ikeda, Davidson, & Zhong, 2012), and liposomes (Liolios, Gortzi, Lalas, Tsaknis, & Chinou, 2009) have been used for the encapsulation of thymol. Films, nanoparticles, and liposomes have been used to deliver nisin (Prombutara, Kulwatthanasal, Supaka, Sramala, & Chareonpornwattana, 2012; Xiao, Gömmel, Davidson, & Zhong, 2011). Research in our lab has shown that phytoglycogen octenyl succinate in non-emulsion and emulsion systems can be used to protect nisin (Bi, Yang, Bhunia, & Yao, 2011; Bi, Yang, Narsimhan, Bhunia, & Yao, 2011).

Various types of antimicrobial compound such as carvacrol, thymol, β-resorcylic acid, and caprylic acid have been used to inhibit the growth of pathogens on cantaloupes (Upadhyay, Upadhyaya, Mooyottu, Kollanoor-Johny, & Venkitanarayanan, 2014). Recently, thymol-based emulsions were prepared to wash organic cantaloupes to improve the microbial safety (Zhang, Ma, Critzer, Davidson, & Zhong, 2016). In our lab, we have been studying the impact of delivery system on the efficacies of antimicrobial compounds, using cantaloupe fresh-cut and whole fruit as models. In the current study, the goal was to evaluate the efficacies of nisin and thymol in the presence of emulsion-based delivery systems against *Listeria monocytogenes* V7 and *Salmonella enterica* serovar Typhimurium. Cantaloupe juice was used as a model, since it usually covers the surface of fresh-cut cantaloupe and contains proteases that may negatively affect nisin activity. The overall hypothesis was that, starch octenyl succinate (starch-OS) stabilized emulsion was able to substantially prolong the efficacy of nisin and thymol, either individually or in combination, against both model pathogens.

2. Materials and methods

2.1. Materials

Starch octenyl succinate (starch-OS) was obtained from a food ingredient company. Our analysis showed that it had a degree of substitution (DS) of 0.022 (molar ratio of octenyl succinate groups to glucosyl groups), with weight-average molecular mass of 7.63×10^5 g/mole. Tween-20 was purchased from Sigma Aldrich (St. Louis, MO). Soybean oil was purchased from local grocery store. Nisin powder (2.5% nisin) and thymol (⩾99.5%) were purchased from Sigma Aldrich (St. Louis, MO). Brain heart infusion (BHI) powder and Bacto agar were purchased from BD Biosciences (Franklin Lakes, NJ).

2.2. Thymol and nisin formulations

2.2.1. Thymol and nisin in non-emulsion, aqueous dispersions

Stock solution of thymol was prepared by adding 2.0 g of thymol crystals to 1.0 L of sterile sodium acetate buffer (pH 5.5, 50 mM). The solution was gently stirred in dark for 24 h at 25 °C. Thereafter, the solution was centrifuged at 7800×*g* for 15 min twice to obtain a thymol concentration of 978.3 μg/mL determined using HPLC analysis. Nisin stock solution was prepared by dispersing 1.2 g of nisin powder in 3.00 mL sterile sodium acetate buffer (pH 5.5, 50 mM). The dispersion was slowly agitated on a rotary shaker (Lab-Line, Max/Rotator, USA) for 15 h at 6 °C. The nisin dispersion was centrifuged at 5000×*g* for 10 min to obtain nisin stock solution at 10.0 mg/mL. In order to prepare nisin and thymol combined solution, nisin stock solution was added to thymol stock solution to obtain a final nisin concentration of 400 μg/mL.

2.2.2. Thymol and nisin in emulsions

Starch-OS, soybean oil, thymol, and sodium acetate buffer (50 mM, pH5.5) were used to prepare thymol-containing oil-in-water emulsion. To prepare the aqueous phase, starch-OS was dissolved in the sodium acetate buffer. To prepare the oil phase, thymol was dissolved in soybean oil. Thereafter, the water and oil portions were mixed and subjected to high-speed homogenization (Ultra Turrax, IKA T25 digital, NC, USA) at 18,000 rpm for 1 min. In the next step, high-pressure homogenization (GEA, PandaPlus, Italy) at 10,000 psi for 3 cycles was performed to obtain the final emulsions. In the generated emulsion (stock emulsion), the concentrations of starch-OS and oil were each 10.0 mg/mL. To determine thymol concentration in the stock emulsion, 100 μL emulsion was added to 900 μL ethanol, and the mixture was vortexed for 10 min. Thereafter, the mixture was centrifuged at 12,000×*g* for 15 min to remove precipitated starch materials. The supernatant was used for thymol quantification with HPLC as described later. The thymol concentration in the emulsion was 972.4 μg/mL.

To prepare nisin in emulsion, similar method was followed without thymol addition to oil. Nisin stock solution (10.0 mg/mL) was added to the formed emulsion to achieve 400 μg/mL, and the mixture was incubated at 4 °C for 24 h on a rotary shaker. To prepare emulsion that contained both nisin and thymol, nisin stock solution was added to the thymol-containing emulsion and the mixture was incubated at 4 °C for 24 h. Different emulsions preparations (nisin, thymol, and combination of nisin and thymol) were immediately transferred to 50-mL tubes, capped and protected from light to prevent thymol evaporation and degradation. All formulated emulsions were sterilized in boiling-water bath for 3 min before proceeding with further tests.

2.3. Preparation of cantaloupe juice

Cantaloupe juice was extracted from cantaloupe fruits purchased from a local grocery store. The cantaloupe pulp was separated and crushed with a commercial blender (Waring Inc, USA). The pulp homogenate was centrifuged at 7800×*g* for 20 min (Beckman Coulter, CA). The supernatant was collected as cantaloupe juice, which was filter-sterilized using a 0.2 μm filter (Millipore, MA) and termed "cantaloupe juice".

2.4. Protease activity measurement of cantaloupe juice

A protease fluorescent detection kit (Sigma Aldrich, St. Louis, MO) was used to determine the protease activity of cantaloupe juice. In this procedure, 20 μL of incubation buffer, 20 μL of FITC-casein substrate and 10 μL solution (test, control, or blank) were mixed together. In this assay, the concentration of the control solution varied between 0.3125 to 20.0 μg/mL. The mixtures were incubated at 37 °C for 4 h in the dark. Incubation was performed again at 37 °C for 30 min after adding 150 μL of 0.6 N trichloroacetic acid (TCA). Centrifugation of the mixture was performed at 10,000×*g* for 10 min and supernatant (10 μL) was withdrawn. This supernatant was then mixed with 1.0 mL assay buffer. Aliquots (200 μL) were transferred from this mixture to a black 96-well plate for quantification of protease activity using fluorescence intensity. All measurements were performed in triplicates.

2.5. Antimicrobial efficacy evaluations

Antimicrobial activity of different preparations was measured in two groups: (1) model group (in the presence of depletion factors) and (2) reference group (in the absence of depletion factors). In the model group, proteolytic enzymes in the cantaloupe juice and chamber headspace for evaporation were depletion factors of